The Influence of Stratum Corneum Morphology on Water Permeability

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The stratum corneum (SC) provides the barrier to water loss for the skin of mammals. A significant body of evidence now exists suggesting that extracellular SC lipids are primarily responsible for this barrier. We have measured the permeability (P) and lag-time (T_{lag}) for water vapor transport through the SC and found that P is about 1000 times less than the values obtained for most other lipid membranes. In addition, we have measured the water partitioning into the lipid microdomain of the SC using a differential scanning calorimetry technique. These combined data provide an estimate of the diffusion coefficient (D) and diffusion pathlength (δ). The results show that the intrinsic diffusion of water is comparable to values obtained with other lipid membranes. The value obtained for δ, however, is fiftyfold greater than the sample thickness. These results are interpreted in terms of the unique morphology of the SC, where lipids form an extracellular continuum that is highly tortuous. Thus, the exceedingly low permeability of the SC may be due, in large part, to its unique morphology. *J Invest Dermatol 96:495–499, 1991*

Although all life forms depend upon membrane-like structures for their existence, nowhere is that more dramatically demonstrated than in the skin. This is particularly true in higher animals, in which this complex membrane has evolved to a multifunctional organ. In terrestrial mammals, one of the skin's most vital functions is to regulate the amount of water lost to the environment. The barrier to water loss resides in the stratum corneum (SC), the thin, outermost layer of the skin with a very unique lipid composition and morphology [1,2]. The SC consists of several layers of keratinized cells (corneocytes) suspended in an extracellular lipid matrix. The lipids of the SC are composed primarily of free fatty acids, ceramides, and cholesterol arranged in multiple lamellae. Recent evidence has shown that a layer of ceramides is covalently bound to the surface of the corneocyte [3]. These bound lipids have alkyl chains of 30 to 34 carbons length, and contain no cholesterol.

The SC has a water permeability about 1000 times lower than most other biomembranes. There exists a wealth of experimental evidence suggesting that SC lipids are responsible for the barrier to water loss [1,2,4,5]. Hence, the exceptionally low water permeability of the SC could reflect its unique lipid composition. Results obtained with simpler membrane systems, however, suggest that changes in lipid composition result in, at most, a tenfold difference in permeability [6,7]. These results were obtained with phospho-, spingo-, and glycero-lipids, suggesting that head group composition has relatively little effect on permeability. Interestingly, however, all of these lipids were of similar alkyl chain length and conformation (i.e., solid or "gel" state). Recent biophysical results from our laboratory have shown that SC water permeability is highly correlated with measures of the lipid alkyl-chain conformation [4,5]. Furthermore, these results were consistent with a model proposed for water permeability through phospholipid membranes [8]. Because SC lipids and phospholipids share a common hydrocarbon domain (but disimilar polar regions), these results suggest that water permeability through lipid membranes is regulated by the alkyl domain. Thus, it seems unlikely that lipid composition alone can account for the exceptionally low water permeability of the SC. Alternatively, the SC has a very unique morphology, by which lipids form multi-lamellar arrays in the extracellular spaces surrounding the corneocytes [1,2,9]. This morphology is analogous to highly impermeable barriers formed by the inclusion of flakes in a homogeneous matrix [9,10] and thus could account for the SC's low permeability.

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Abbreviations:
D: diffusion coefficient
DSC: differential scanning calorimetry
δ: diffusion pathlength
HTO: tritiated water
K: partition coefficient for water into the SC
P: water vapor permeability
rh: relative humidities
SC: stratum corneum
T_{lag}: lag time associated with water vapor permeability
T_m: midpoint of the thermal transition

METHODS

Stratum Corneum Preparation The SC was obtained from porcine skin via a technique described previously [11]. Briefly, full-thickness thoracic skin was removed from domestic pigs within a few minutes of sacrifice. These samples were transported to the laboratory on ice where they were trimmed to a uniform depth of 350 μm using a dermatome (Padgett, Kansas City, MO). Pieces of skin, cut to about 5 × 5 cm squares, were incubated at room temperature, SC side up, on filter paper saturated with a 1% solution of trypsin (Sigma, St. Louis, MO) in buffer. Following several hours of enzymatic digestion of the underlying tissue, the SC was peeled away with forceps. This tissue was thoroughly washed, and then dried and stored in a desiccator until use.

Differential Scanning Calorimetry (DSC) Calorimetric data were obtained as described previously [11]. In short, about 10 mg (dry weight) samples of SC were equilibrated for several days at 22°C in an atmosphere of the desired relative humidity above the appropriate saturated salt solution. Samples were then sealed in a

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Table I. Changes in the T_m of Lipid-Associated Peaks in Porcine SC as a Function of Relative Humidity[a]

T_{m1} (°C)	T_{m2} (°C)	Saturated Solution	Mole Fraction of Water in the Solution	Relative Humidity (%)
67.5 ± 0.7	79.2 ± 0.5		0	0
63.1 ± 0.3	73.8 ± 0.3	NaCl	0.9	75
61.8 ± 0.4	72.3 ± 0.4	K_2SO_4	0.99	95

[a] T_m values represent mean ± SEM of 10 determinations.

water-tight cell and heated in the DSC (MC-2, MicroCal, Amherst, MA) from 20 to 120°C at 0.75°C/min.

Flux of Tritiated Water (HTO) Vapor The methods used to measure HTO permeability through porcine SC have been previously described [4]. The water vapor permeability was measured in order to avoid unstirred layer effects that often complicate liquid-phase experiments. In brief, a sheet of porcine SC was mounted between two halves (donor and receiver chamber) of a diffusion apparatus. A reservoir in each half chamber contained 2 ml of a saturated aqueous solution of NaCl. The sample, in contact with the water vapor above these solutions (75% relative humidity), was allowed to hydrate for several days prior to the experiment. To initiate the permeability experiment, 10 μl of HTO (New England Nuclear, 1 μCi/μl) was introduced through a septum into the NaCl solution in the donor chamber, where the vapor was in contact with the outer surface of the SC. Ten-microliter aliquots were removed periodically from the solution in the receiver chamber. The amount of ^3H in each sample was determined by liquid scintillation counting. After an initial lag phase of several hours, the amount of HTO in the receiver chamber increased linearly with time. The steady-state rate of HTO transport through the sample was determined from a linear regression analysis of these data. This rate, when divided by the surface area of the sample (1 cm^2) and the concentration of the permeant (HTO) in the donor chamber, yielded the permeability (P) of water vapor transport through porcine SC. In addition, extrapolation of the steady-state line to the time axis provided an estimate of the lag-time (T_{lag}).

RESULTS

The water vapor phase permeability (P) and lag-time (T_{lag}) were measured for samples of porcine SC equilibrated at 75% relative humidity and 22°C. The P and T_{lag} values obtained for samples from 10 different animals, each measured in triplicate, were $1.1 \pm 0.1 \times 10^{-7}$ cm/sec and $8.4 \pm 1.0 \times 10^3$ sec, respectively. The water permeability is in good agreement with values obtained for porcine [12] and human [13] SC. In contrast, the water permeability through lipid bilayer membranes is more than 1000 times greater [6,7].

The DSC thermal profiles were obtained for porcine SC samples hydrated at 22°C and at various relative humidities (rh). In particular, SC samples were equilibrated at either 0% (dessicant), 75% rh (saturated NaCl), or 95% rh (saturated K_2SO_4). The transition temperatures (T_m) were determined for the transitions near 65°C (T_{m1}) and 75°C (T_{m2}). The results are shown in Table I.

DISCUSSION

Following the arguments put forth by Stein [14] where the lipid hydrocarbon interior was considered homogeneous and rate-limiting, the permeation of water through lipids is a solubility-diffusion process described by Eq. (1),

$$P = KD/\delta. \quad (1)$$

Thus, the permeability can be affected by changes in the partitioning (K), diffusion (D), and/or pathlength (δ). In addition, the lag-time is related to D and δ by Eq. (2).

Equation (1) has been used to analyze the permeability of heterogeneous, mixed-lipid systems at temperatures well away from the lipid phase-transition temperature [14]. In these systems, all lipids were in a gel or solid state, and thus, although compositionally heterogeneous, the lipid hydrocarbon interior was homogeneous. Similarly, the experiments reported here were performed at a temperature more than 40°C below the lipid solid-to-liquid transitions noted in these samples. Thus, Eq. (1) can be applied to lipid systems even as heterogeneous as porcine SC, provided that the studies were performed under conditions where all lipid hydrocarbon chains are in the same physical state (e.g., gel),

$$T_{lag} = \delta^2/(6D). \quad (2)$$

Some investigators have measured P and the bulk partitioning of water by the SC in order to deduce D and δ using Eqs. (1) and (2) [13]. Because this partitioning reflects contributions of proteins and lipid domains, its use in these calculations may be inappropriate. The value of K for water partitioning into the lipid domain, however, can be evaluated from calorimetric results. Porcine and human SC are characterized by a number of thermal transitions that have been detected by differential scanning calorimetry [11,15]. Porcine SC exhibited three transitions near 65, 75, and 100°C, and the two lowest temperature transitions were unequivocally associated with extracellular lipids [11,15]. Furthermore, like many lipid systems, the transition temperature (T_m) was lowered with increasing SC water uptake [11]. The results presented in Table I show that the T_m for both transitions was lowered when samples were hydrated in atmospheres of increased relative humidity. In contrast, the transition enthalpy (ΔH), as measured by the baseline-corrected area under each transition, was unaffected by these changes in water content, with values of 0.4 ± 0.1 and 0.3 ± 0.1 cal per g of SC ($n = 9$), respectively [16]. This enthalpy is in good agreement with the value obtained for human SC [17]. Furthermore, total lipids extracted from porcine SC had a ΔH value of 5.0 cal per g of lipid. These values are quite consistent given that the SC is about 15% lipid, by mass [18]. Finally, assuming an average molecular weight of about 500 for SC lipids [1,2], the molar enthalpies (ΔH_0) were about 1.3 and 1.1 kcal/mol for the transitions near 65 and 75°C, respectively.

At low water concentration, the temperature of a lipid transition is lowered according to freezing-point depression theory,

$$\Delta T_m = -(RT_0^2/\Delta H_0)\, X_{H_2O,lipid}. \quad (3)$$

Thus, T_m is lowered ($\Delta T_m = T_0 - T_m$) in proportion to the mole fraction of water in the lipid membrane ($X_{H_2O,lipid}$), where R is the gas constant, and T_0 is the transition temperature of the pure lipid. Equation (3) assumes an ideal system in which the solute (water) and solvent (lipid) do not interact with themselves or each other. Many systems exhibit "ideal" behavior at low solute concentrations. For example, the T_m for phospholipid membranes decreased linearly with increasing alkanol concentration in the external medium [20]. Similarly, in the experiments reported here, T_m decreased linearly with increasing relative humidity of the external atmosphere. Therefore, in the water-concentration range investigated, SC appears to behave "ideally."

Because SC samples were equilibrated in the vapor above saturated salt solutions, the water concentration in the lipid and solution are related by the partition coefficient K defined by Eq. (4),

$$K = X_{H_2O,lipid}/X_{H_2O,\,solution}. \quad (4)$$

Substituting Eq. (4) into (3) yields

$$\Delta T_m = -(RT_0^2/\Delta H_0)\, K\, X_{H_2O,solution}. \quad (5)$$

From the known saturation concentrations of aqueous NaCl (75% rh) and K_2SO_4 (95% rh) solutions [19], the mole fraction of water in each solution was calculated. These values are shown in Table I. A linear regression analysis of the T_m vs $X_{H_2O,\,solution}$

Table II. Lipid Transition Temperature T_0 and Water-Lipid Partition Coefficient Determined from a Linear Regression Analysis of the Data in Table I[a]

Lipid Transition	T_0 (°C)	Partition Coefficient ($\times 10^2$)
T_{m1}	67.5 ± 0.1	5.9 ± 0.8
T_{m2}	79.2 ± 0.1	6.7 ± 0.9

[a] Error estimates were determined from the regression analysis.

data yielded a slope, which was proportional to K, and an intercept proportional to T_0. Those values, along with error estimates from the linear regression analysis, are shown in Table II. The K values were the same, within error, for both transitions (average value of 6.3×10^{-2}), and reflect the partitioning of water into the lipid domain. A similar technique has been used to evaluate the partitioning of anesthetics into lipid bilayers [20]. One potential problem with these results is that the samples were equilibrated at room temperature, whereas changes in T_m were measured at about 60°C. We have previously shown, however, that water uptake by porcine SC and extracted SC lipids maintained at 75% rh does not change from 20 to 60°C [21]. Similarly, Spencer et al showed that water uptake by human SC was independent of temperature for samples hydrated at high rh [22]. Thus, uptake results obtained by DSC techniques accurately reflect values at lower temperatures.

The K value presented here is greater than the partitioning of water in low-molecular-weight polyethylene [23] and alkanes [24], but less than the value obtained in phospholipid bilayers [25], suggesting that SC lipids have a polarity between these two extremes. The experimental value is also similar to the water-solvent partition coefficient obtained for octanol but much larger than the value obtained with hexadecane [14]. The similarity between the water partition values obtained with octanol and the SC is in agreement with the results of Anderson et al [18], who suggested that these two media exhibited similar uptakes of polar hydrogen-bonding groups (e.g., —OH). In contrast, the partition value measured here is about 100 times less than that measured for the bulk uptake of water by the SC [13,21], most likely due to the large influence of proteins in those latter measurements.

Substitution of the experimental values for P, T_{lag}, and K into Eqs. (1) and (2) yielded values of 880 μm and 1.5×10^{-7} cm²/sec for δ and D, respectively. Given the experimental errors associated with each value (about 10% relative error), and the assumptions of Eqs. (1)–(5), these values represent an "order-of-magnitude" calculation. By comparison, some investigators have used P, the bulk uptake of water by the SC, and the sample thickness to deduce D using Eq. (1) [13]. The value derived was several orders of magnitude less than the D presented here. However, because the bulk uptake of water by the SC reflects contributions of both the protein and lipid domains, Eq. (1) is inappropriate for such calculations.

A large body of evidence suggests that water permeability through the SC is governed by extracellular lipids. First, lipids form the only continuous domain within the SC [1,2]. Water molecules, therefore, ultimately must traverse the lipid domain in order to permeate through the sample. Second, removal of SC lipids resulted in a dramatic increase in water permeability [26]. In addition, an analysis of the change in SC water permeability with temperature yielded an activation energy equivalent to that obtained for lipid bilayers [4]. Finally, results obtained using infrared spectroscopy showed a strong correlation between temperature-induced changes in SC lipid acyl chain disorder and water permeability [5]. Similarly, theoretical predictions [8], as well as experimental measurements with water [7,8] and other non-electrolytes [27], have shown that permeability through membranes is related to increased mobility of the lipid hydrocarbon chains. Taken together, these results strongly suggest that extracellular lipids represent the rate-limiting domain to SC water permeability. The kinetic values (T_{lag} and P) measured experimentally, therefore, most likely reflect the properties of this rate-limiting domain.

The value of δ derived in these experiments is about 50 times greater than the sample thickness (about 15 μm), suggesting that water traversed a tortuous path during its movement across the SC. The δ value here is of the same order of magnitude as the value obtained by Albery and Hadgraft for the permeation of methyl nicotinate through human SC [28]. Perhaps the most compelling supportive evidence comes from the electron spin resonance (ESR) study of molecular oxygen diffusion through SC by Plachy and Hatcher [29]. Due to the paramagnetism of O_2, it will increase the ESR linewidth of a spin probe in a manner dependent upon the product of the oxygen solubility and diffusion coefficient. Spin probes were selectively incorporated into SC lipid and changes in ESR linewidth were monitored as O_2 permeated through the sample. The results showed that the diffusion coefficient determined in intact porcine SC was about 300 times less than the value obtained for isolated lipids. These results strongly suggest that oxygen diffuses through the SC via a tortuous extracellular (e.g., lipid) pathway.

It appears unlikely that the approximately 1000-times lower SC water permeability relative to other lipid membranes is related solely to its unique lipid composition. As discussed above, the water permeability through lipid membranes is controlled primarily by hydrocarbon domains. Although differing significantly in the polar region, the SC lipids have long saturated hydrocarbon chains [1,2], similar to those of many common biomembranes. Furthermore, measurements made on model systems suggest that changes in lipid composition alter water permeability by no more than about tenfold [7,8]. Thus, there is no compelling reason to believe that the lipid barrier formed by the SC should be inherently less permeable than other biomembranes.

An alternative hypothesis for the low permeability of the SC can be derived from a barrier membrane theory derived by Cussler et al [10]. They demonstrated that the incorporation of impermeable flakes into a homogeneous medium can reduce the permeability by orders of magnitude compared to the pure phase. The SC has a similar construction of corneocyte "flakes" incorporated into a lipid "matrix." If one assumes that the corneocytes are impermeable square blocks with a thickness of 0.5 μm and width of 30 μm, and are spaced 0.1 μm apart [30], then according to Cussler's theory [9] the permeability will be reduced by about 1000 times relative to a pure lipid phase. Similarly, Michaels et al [9] calculated the permeability due to transport via a lipid pathway surrounding brick-like corneocytes. Using dimensional values for the corneocytes similar to those above, their results suggest that transport solely through the SC lipid phase reduces the permeability by about 1000 times relative to transport through a homogenous lipid domain. These order-of-magnitude calculations are in good agreement with the results presented here, showing that water permeability through the SC is about 1000 times less than obtained with other lipid biomembranes. Finally, in both of these theoretical descriptions of transport via a lipid pathway, the results predict that permeability should decrease with increasing size of the corneocytes. Results obtained with human volunteers have shown that there is an inverse relationship between increased corneocyte size and decreased water loss [31]. These results suggest that the unique morphology of the SC may impart a tortuosity to water transport that is important to its barrier properties.

At first glance, the assumption of water-impermeable corneocytes appears unlikely given the high water uptake of the SC [32]. The experiments described here, however, were done under conditions (75% rh) in which water in the SC is tightly bound [33], most likely to protein components of the corneocytes. Thus, although corneocytes may act as a "sink" for water, this water may not be available for net transport, especially under these experimental conditions. As a result, the corneocytes may be water-impermeable on a time-scale appropriate to water transport. Results obtained for aqueous proton diffusion in the SC support this hypothesis [34]. In

those studies, the diffusion of water-associated protons was measured in guinea pig foot pad SC using proton magnetic resonance (PMR) relaxation techniques. This technique (like the ESR experiments described above) has the advantage that results are obtained on a time-scale that is relevant to diffusion within the sample. The PMR data were analyzed using a barrier plane model of the SC. Results showed that transport normal to the surface of the SC was restricted to a few μm, whereas diffusion parallel to the surface was much less spatially constrained. These diffusional constraints were associated with the corneocyte membrane. In both directions, however, D was about 10^{-6} cm^2/sec for highly hydrated samples, and decreased with decreasing water content of the sample. In addition, treatment of the SC with a lipid solvent substantially reduced the diffusion barrier, especially in a direction normal to the surface. From these results the investigators concluded that on a time-scale relevant to diffusion of water-associated protons, the corneocyte was impermeable. Stated alternatively, water-associated protons were constrained to the extracellular spaces where they had a diffusion coefficient similar to that seen in other lipid membrane systems.

In addition to retarding transport by acting as a sink for water, a relatively impermeable corneocyte could result from the covalently attached lipids that line the outside of the cell membrane [3]. These lipids have exceptionally long alkyl chains (30–34 carbons) and contain no cholesterol. Furthermore, ESR results have shown that these bound lipids are more hydrophobic than other SC lamellae [35]. All of these properties are consistent with a relatively impermeable lipid layer surrounding the corneocytes.

The results from a number of studies using model and/or reconstituted SC supports the barrier hypothesis proposed above. For example, several researchers have reconstituted human SC from isolated corneocytes and solvent-extracted lipids [36,37]. In each case, the permeabilities of the reconstituted and initial SC were similar. More recently, Abraham and Downing have shown that SC lipids added to isolated porcine corneocytes form lamellar sheets attached to the extracellular surface of the cells [38]. Thus, the multi-lamellar lipid structure appears to be maintained in these reconstituted systems. Furthermore, Friberg et al have shown that a variety of model lipids can be used to reaggregate corneocytes into a film with permeability properties quite similar to the SC [39]. These results lead the authors to conclude that the "lipid barrier to water penetration through the SC is determined by the structural organization of the lipids, not by the exact chemical structure of the individual species." Finally, Friberg and Kayali measured the rate of water evaporation from a uniform layer of these model lipids [40]. Analysis of these results in terms of water diffusion from a uniform slab lead to a value for D of about 1×10^{-7} cm^2/sec at 80% rh, in excellent agreement with the results presented here and similar to water diffusion through a number of lamellar lipid membranes. Although these results can be criticized for the use of model lipids of questionable relevance to the SC, these results suggest that corneocyte "inclusions" into the SC lipids serve to dramatically lower water permeability. In fact, these results argue that as far as water permeability is concerned, the precise lipid composition of the SC may be irrelevant.

Although the experimental results presented here suggest that lipid tortuosity reduces water transport through the SC, these results may have broader application. For example, similar conclusions have been derived for the transport of O_2 [29], methyl nicotinate [28], and water-associated protons [34] through the SC. Furthermore, Hadgraft and Rideout measured the transport of a number of different drugs through human SC and a model membrane made of a porous filter impregnated with isopropyl myristate [41]. Their results showed that transport through skin and the model membrane were highly correlated for six drugs with octanol/water partition coefficients ranging from about 4 to 200. More importantly, transport through the model membrane was uniformly about 1000 times greater than through the SC, suggesting that permeation via lipid pathways within the SC was highly tortuous. One might argue that the results obtained for lipophilic compounds such as methyl nicotinate, or even O_2, may have little relevance to the transport of a small, polar molecule such as water. However, Rougier et al [31] have shown that water and benzoic acid permeability are highly correlated among a group of human subjects. Zatz (personal communication) has found a similar correlation between the in vitro skin permeability of lidocaine and water. These correlations suggest that the transport of water and lipophilic compounds share a common lipid pathway. The high tortuosity noted for the SC permeability of water and larger, lipophilic permeants may similarly reflect a common pathway.

In conclusion, the results presented here suggest that water vapor permeability through porcine SC is characterized by an intrinsic diffusion coefficient that is similar to other lipid membranes. In conjunction with other biophysical results, the results presented here strongly support the role of lipids in SC water barrier function. The exceptionally low permeability of the SC, however, is not necessarily due to the unique lipid composition. Rather, the unique morphology of the SC results in a highly convoluted and tortuous lipid pathway for water diffusion. As a consequence, water molecules must migrate over much greater distances than the sample thickness.

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